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Abstract: Sexual harassment by males has been reported from several live-bearing fishes (Poeciliidae) and has been shown to inflict costs on females. For example, poeciliid females have reduced feeding opportunities when accompanied by a male because females dedicate attention to avoiding male copulation attempts. Poeciliid species differ considerably in male mating behavior, such as the presence or absence of courtship. Courting males display in front of the females, but males attempting to sneak-copulate approach females from behind, that is, in the blind portion of their visual field, and force copulations, which can be viewed as a male persistence trait. We predicted that poeciliid females need to be more vigilant in the presence of noncourting males, and costs of harassment by noncourting males might be stronger. In a comparative approach we examined the costs of male sexual harassment for females as reduced feeding time in 9 species of live-bearing fishes, including courting (Poecilia latipinna, Poecilia reticulata, Xiphophorus cortezi, Xiphophorus variatus) and noncourting species (Poecilia mexicana [surface- and cave-dwelling form], Poecilia orri, Gambusia affinis, Gambusia geiseri, Heterandria formosa). In all species examined except for the cave form of P. mexicana, focal females spent significantly less time feeding in the presence of a male than when together with another female. The time females spent feeding was found to significantly decline with increasing male mating activity (sum of all sexual behaviors), but there was no support for the idea that females would spend more time feeding in the presence of courting males compared with noncourting ones.

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Sexual harassment in live-bearing fishes (Poeciliidae): comparing courting and noncourting species

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Sexual harassment by males has been reported from several live-bearing fishes (Poeciliidae) and has been shown to inflict costs on females. For example, poeciliid females have reduced feeding opportunities when accompanied by a male because females dedicate attention to avoiding male copulation attempts. Poeciliid species differ considerably in male mating behavior, such as the presence or absence of courtship. Courting males display in front of the females, but males attempting to sneak-copulate approach females from behind, that is, in the blind portion of their visual field, and force copulations, which can be viewed as a male persistence trait. We predicted that poeciliid females need to be more vigilant in the presence of noncourting males, and costs of harassment by noncourting males might be stronger. In a comparative approach we examined the costs of male sexual harassment for females as reduced feeding time in 9 species of live-bearing fishes, including courting (*Poecilia latipinna*, *Poecilia reticulata*, *Xiphophorus cortezi*, *Xiphophorus variatus*) and noncourting species (*Poecilia mexicana* [surface- and cave-dwelling form], *Poecilia orri*, *Gambusia affinis*, *Gambusia geiseri*, *Heterandria formosa*). In all species examined except for the cave form of *P. mexicana*, focal females spent significantly less time feeding in the presence of a male than when together with another female. The time females spent feeding was found to significantly decline with increasing male mating activity (sum of all sexual behaviors), but there was no support for the idea that females would spend more time feeding in the presence of courting males compared with noncourting ones. *Key words:* courtship, *Gambusia*, mating tactics, *Poecilia*, sexual conflict, *Xiphophorus*. *[Behav Ecol 18:680–688 (2007)]*

In polygamous species without paternal care, males are predicted to attempt to maximize their inclusive fitness by mating with as many females as possible (Bateman 1948; Trivers 1972). Males may further attempt to maximize their shared paternity by showing high numbers of copulations even toward the same female to increase the number of transferred sperm (Parker 1998; Evans et al. 2003). Sexual conflict between the sexes may arise due to opposed interests regarding quantity and quality of mates (Parker 1979; Smuts BB and Smuts RW 1993; Rowe et al. 1994; Parker and Partridge 1998; Chapman et al. 2003; Pizzari and Snook 2003; Arnqvist and Rowe 2005).

In their book on sexual conflict, Arnqvist and Rowe (2005) argued that reproductive competition among males leads to the evolution of traits that increase male persistence and that more persistent males impose fitness costs for females. Female traits that reduce the costs imposed by persistent males are then favored by selection (resistance traits), promoting again the evolution of novel male traits that increase reproductive competitive abilities in males. According to this model, the sexes are locked in a constant evolutionary arms race in which male persistence and female resistance traits coevolve (Arnqvist and Rowe 2005). A different view was expressed by Cordero and Eberhard (2003), who argued that male persistence—although costly for females—may actually be a sexually selected trait because females obtain a fitness benefit by having more persistent sons (but see Cameron et al. 2003). Thus, to determine if a given male persistence trait is detrimental for the female's fitness, future studies will not only need to determine the costs of male persistence for females but also need to establish that these costs actually outweigh potential indirect benefits.

Female costs of interacting with males may vary considerably if males embark on different tactics in order to achieve fertilization success. Different male mating tactics are present in many species; for example, whereas some males try to monopolize resources or females in order to maximize their fertilization success (bourgeois tactic), others use alternative mating tactics like sneaky copulations (Taborsky 1994; Gross 1996; Birkhead and Møller 1998). Given a choice, females usually prefer to mate with bourgeois males (Taborsky 1994). In the context of sexual conflict and the coevolution of persistence and resistance between the sexes, it has been hypothesized that different male tactics raise different levels of fitness costs for females (Arnqvist and Rowe 2005). However, tests of this idea are as yet very scarce. In this study, we use different species of live-bearing fishes (Poeciliidae), in which male mating tactics vary both within and between species, in order to evaluate female costs in dependence of male mating tactics.

Poeciliid fishes are prime examples to illustrate the sexual conflict resulting from differential interests of the sexes concerning the number of copulations (Magurran and Seghers 1994; Houde 1997; Magurran 2001; Brewster and Houde 2003). Poeciliids reproduce via internal fertilization and ovovivipary, and males use their modified anal fin, the gonopodium, as a copulatory organ (Rosen and Bailey 1963; Greven 2005). Parental care is lacking, and poeciliid mating systems

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Table 1
The mean (±SE) time focal females of the 9 species spent feeding when interacting with another female or a male

	Origin	Time spent feeding with female (s)	Time spent feeding with male (s)	Difference in feeding times, paired *t*-test
Sailfin molly, *Poecilia latipinna*	Lafayette, LA	76.6 ± 17.3	35.8 ± 9.5	$t_{19} = 2.10$, $P =$ **0.049**
Sailfin molly, *Poecilia latipinna*	Weslaco, TX	35.8 ± 11.9	19.3 ± 6.3	$t_{11} = 2.57$, $P =$ **0.026**[a]
Atlantic molly, *Poecilia mexicana*	Tampico, Tamaulipas, Mexico	103.3 ± 16.7	46.6 ± 11.4	$t_{23} = 4.45$, $P =$ **0.0002**
Cave molly, *Poecilia mexicana*	Cueva del Azufre, Tabasco, Mexico	56.1 ± 13.5	58.7 ± 12.1	$t_{25} = -0.82$, $P = 0.42$[a]
Mangrove molly, *Poecilia orri*	Roatan Island, Honduras	3.6 ± 1.1	3.4 ± 2.4	$t_{19} = 2.82$, $P =$ **0.011**[a]
Guppy, *Poecilia reticulata*	San Antonio, TX (feral)	53.5 ± 9.9	37.2 ± 9.0	$t_{19} = 2.14$, $P =$ **0.045**
Western mosquito fish, *Gambusia affinis*	San Marcos River, TX	161.3 ± 14.3	93.5 ± 16.7	$t_{14} = 3.68$, $P =$ **0.0025**
Largespring mosquito fish, *Gambusia geiseri*	San Marcos River, TX	44.5 ± 14.5	14.4 ± 6.8	$t_{14} = 2.97$, $P =$ **0.010**
Least killifish, *Heterandria formosa*	New Orleans, LA	14.8 ± 6.0	6.9 ± 3.2	$t_{15} = 3.42$, $P =$ **0.004**[a]
Delicate swordtail, *Xiphophorus cortezi*	Río Panuco, San Luis Potosi, Mexico	28.0 ± 10.9	5.8 ± 1.7	$t_{11} = 2.37$, $P =$ **0.037**[a]
Variable platyfish, *Xiphophorus variatus*	Arroyo Zarco, Tamaulipas, Mexico	138.6 ± 17.9	77.7 ± 15.2	$t_9 = 2.97$, $P =$ **0.016**
Variable platyfish, *Xiphophorus variatus*	Encino, Tamaulipas, Mexico	152.8 ± 19.9	83.8 ± 15.2	$t_9 = 3.62$, $P =$ **0.006**

Significant *P*-values ($P < 0.05$) are in bold typeface.
[a] Test on log-transformed data.

are highly promiscuous. Poeciliid females have a roughly monthly sexual cycle (Parzefall 1973), and they can store sperm and thus require few copulations to ensure complete fertilization of several monthly broods (Constantz 1984, 1989). Males, by contrast, almost constantly engage in sexual behavior (e.g., guppy, *Poecilia reticulata*: Magurran and Seghers 1994; Godin 1995; Houde 1997; Magurran 2001; Atlantic molly, *Poecilia mexicana*: Plath et al. 2003, 2005; and mosquito fish, *Gambusia holbrooki*: Bisazza and Marin 1995). Guppy females are receptive for less than 5% of days in their reproductive cycle just after parturition (Houde 1997; see also Farr and Travis [1986] for *Poecilia latipinna*). Guppy females cooperatively copulate only 2 or 3 times (Kelly et al. 1999) and typically flee from approaching males (e.g., Brewster and Houde 2003) when they are not receptive or virgin (Liley 1983).

In live-bearing fishes, where males show mating behavior almost constantly, females need to dedicate considerable time to avoid unwanted copulations, which may induce shifts in female behavior (e.g., *G. holbrooki*: McPeak 1992; Pilastro et al. 2003; Dadda et al. 2005; Agrillo et al. 2006). Several studies have reported on costs for females arising from this male sexual harassment in terms of a reduction of female feeding efficiency in the presence of a harassing male (Poeciliidae: guppy, *P. reticulata*: Magurran and Seghers 1994; Griffiths 1996; sailfin molly, *P. latipinna*: Schlupp et al. 2001; Atlantic molly, *P. mexicana*: Plath et al. 2003; mosquito fish, *G. holbrooki*: Pilastro et al. 2003; Goodeidae: dark-edged split-fin, *Girardinichthys multiradiatus*: Valero et al. 2005). Access to food strongly predicts females' growth and fecundity in poeciliids (guppy: Hester 1964; Reznick 1983; Reznick and Miles 1989; mosquito fish *Gambusia affinis*: Tobler M, unpublished data). Therefore, it seems very likely that reduced feeding opportunities by male harassment represent a cost for females (Magurran and Seghers 1994).

Poeciliid males show pronounced differences in mating behavior (Farr 1989; Bisazza 1993). In some poeciliid species, such as least killifish (*Heterandria formosa*; Farr 1989; Bisazza and Pilastro 1997), mosquito fish (*Gambusia* spp.; Pilastro et al. 1997), or the short-fin mollies *P. mexicana* (Atlantic molly; Parzefall 1969; Ptacek 2002; Plath et al. 2003; MacLaren and Rowland 2006) and *Poecilia orri* (mangrove molly; Farr 1989), males exclusively rely on sneaky copulations without prior courtship. Courtship and sneaky mating attempts can also occur as facultative, that is, context-dependent mating tactics (e.g., guppy: Baerends et al. 1955; Godin 1995; Houde 1997; Magurran and Seghers 1990; Ojanguren and Magurran 2004), or male mating tactics vary with body size (e.g., genus *Xiphophorus*: Ryan and Causey 1989; Zimmerer and Kallman 1989; Ryan et al. 1990, 1992; *P. latipinna*: Parzefall 1969; Woodhead and Armstrong 1985; Farr et al. 1986; Travis 1994; Ptacek and Travis 1996; Ptacek 2002; guppy: Reynolds et al. 1993; Magellan et al. 2005). For example, large, ornamented *P. latipinna* males use their enlarged, sail-like dorsal fin for visual displays, whereas smaller males have a female-like morphology and rely more on sneaky mating tactics (Parzefall 1969; Woodhead and Armstrong 1985; Farr et al. 1986; Travis 1994; but see also Travis and Woodward [1989] for context-dependent mating behavior in *P. latipinna*). Consequently, poeciliids are excellent models to study alternative mating behavior because variation in male mating tactics can be found not only among species but also among different male phenotypes within a species or even within phenotypically similar males depending on context.

Poeciliid males typically court within the visual field of the female, whereas males attempting to sneak-copulate approach the female from behind, that is, in the blind portion of her visual field (e.g., Parzefall [1969] for *Poecilia*; Farr [1989] and Pilastro et al. [1997] for *Gambusia*; Farr [1980a] for metallic live-bearer, *Girardinus metallicus*). Therefore, a female attempting to avoid unwanted copulations might need to dedicate less vigilance to a courting male compared with a male employing sneaky mating (Pilastro et al. 2003 for a discussion). If male coercion is viewed as a male persistence trait (as suggested by Arnqvist and Rowe 2005; see also Schlupp et al. 2001; Pilastro et al. 2003 for discussions), it can be predicted that females should suffer higher fitness costs when interacting with males embarking on a coercive (sneaky) mating tactic rather than courtship. We tested this prediction using 9 species of poeciliid fishes that show pronounced variation in male mating tactics, that is, the presence or absence of courtship and sneak mating. We examined the link between the overall number of sexual behaviors of males, the relative frequency of courtship displays, and the reduction of female feeding time by male harassment.

METHODS

Study organisms and fish maintenance

We studied sexual harassment in 9 species of poeciliid fishes, including the genera *Poecilia*, *Gambusia*, *Heterandria*, and *Xiphophorus* (Table 1). In the case of sailfin mollies (*P. latipinna*)

and variable platyfish (*Xiphophorus variatus*) 2 different populations per species were examined. In the case of the Atlantic molly (*P. mexicana*) we studied both a typical surface-dwelling population and the only described cave-dwelling poeciliid, the cave molly, a subterranean form of *P. mexicana*, which differs from epigean Atlantic mollies in a variety of characters like reduced, but functional eyes and reduced pigmentation (Gordon and Rosen 1962; Parzefall 2001). Guppies (*P. reticulata*) came from a feral population in San Antonio, TX. Origins of the different species/populations are summarized in Table 1.

The delicate swordtails (*Xiphophorus cortezi*) were maintained in outdoor breeding tanks (2500 l) at the Brackenridge Field Laboratory of the University of Texas at Austin. The 2 lineages of variable platyfish (*X. variatus*) were obtained from the *Xiphophorus* Genetic Stock Center, Texas State University in San Marcos, TX. All other species/populations were maintained as large, randomly outbred stocks in tanks (1000 l) in a greenhouse of the University of Oklahoma in Norman. The tanks contained naturally growing algae and other submerged plants as well as a variety of naturally occurring invertebrates like chironomid larvae, copepods, and amphipods, on which the fish could feed. In addition, the fish were fed ad libitum amounts of flake food every 2 days. All fish used in this study were sexually mature and had interacted with the opposite sex; thus, all females were most likely pregnant.

All fish were acclimated to laboratory conditions for 24 h and were fed food tablets, making sure that the fish would be habituated to and feed on food tablets during the tests. Then, males were isolated from females in small, visually separated aquaria for another 24 h. Meanwhile, the focal females were isolated in small groups (4–5 individuals) in 50-l aquaria and were not fed for 24 h before the tests, ensuring that they were motivated to feed throughout the test. Males and partner females were fed ad libitum in the morning just prior to the tests, making sure that males would not trade off foraging and mating (Abrahams 1993).

Feeding experiment I: comparison of different species

For the feeding experiments, we used 3 identically equipped test tanks in random order. The test tanks (50 × 30 × 25 cm length × height × width) were filled to three-fourths with aged tap water. The base was covered with a 1-cm layer of white gravel. Water temperature was maintained at 25.2 ± 0.1 °C (mean ± SE) with the aid of an aquarium heater. Illumination was provided by four 60-W lamps on the ceiling of the test room.

Prior to a feeding test, a food tablet (TetraMin tropical tablets) was placed on the bottom, centrally near the front wall. A transparent Plexiglas box (30 × 8 × 8 cm, open at the top and base) was placed in the back center of the tank to hold the focal female during the acclimation phase. To initiate a trial, a focal female was introduced into the acclimation box, and a male or a female partner fish was introduced into the test tank. Both fish were given 5 min of acclimation. We then released the focal female, and the behavior observation was started. We measured the time the focal female spent feeding from the presented stationary food source, from the surface of the water, the aquarium walls, and bottom, and on floating matter during a during 5-min observation period. When a male partner was present, we also counted the number of sexual behaviors. Because the different species are known to show differences with respect to male mating behavior (Parzefall 1969; Farr 1989; Greven 2005), male sexual behavior was recorded in 3 encompassing categories: 1) premating behavior involving body contact (e.g., nipping at the female gonopore), 2) gonopodial thrusts (copulations and copulatory attempts), and 3) courtship behavior (e.g., sigmoid displays in guppies). Nipping at the female genital pore typically precedes copulation attempts in several species, for example, mollies (Parzefall 1969, 1973; Sumner et al. 1994).

After the first part of a trial, the focal female was introduced into the acclimation tube again, the first partner was removed, and a partner fish of the opposite sex was introduced. Hence, each focal female experienced 2 subsequent experimental situations during which she could feed either with a male or with another female. The order of presentation (male or female partner first) was balanced. After a trial, all fish involved were measured for standard length to the closest 0.5 mm (Table 2).

We initially planned to test also for an effect of aggressive interactions between females on female feeding times. Therefore, all aggressive interactions between females (as well as between the focal female and the male) were recorded (see Parzefall [1969] for a description of aggressive behaviors in poeciliid fishes). However, aggressive interactions (such as biting, aggressive fin erection, etc.) occurred rarely; in cave

Table 2
Standard lengths (SL) of the test fishes and male sexual behaviors (mean ± SE) in the 9 species examined

	Focal female SL (mm)	Partner female SL (mm)	Male SL (range) (mm)	Precopulatory behavior (nipping)	Copulatory attempts (gonopodial thrusts)	Courtship behavior
Sailfin molly, *Poecilia latipinna* (Lafayette)	32.4 ± 0.8	29.6 ± 1.2	29.9 ± 1.3 (21–42)	12.4 ± 6.1	2.0 ± 1.5	0.3 ± 0.2
Sailfin molly, *Poecilia latipinna* (Weslaco)	36.7 ± 1.7	34.4 ± 1.6	27.5 ± 2.1 (16–43)	8.9 ± 2.4	1.2 ± 0.7	0.3 ± 0.3
Atlantic molly, *Poecilia mexicana*	37.1 ± 1.3	34.9 ± 1.7	31.5 ± 1.0 (23–44)	27.9 ± 7.8	6.0 ± 2.4	0
Cave molly, *Poecilia mexicana*	37.5 ± 1.0	36.6 ± 1.2	30.1 ± 0.7 (23–37)	16.7 ± 3.9	3.8 ± 1.0	0
Mangrove molly, *Poecilia orri*	35.8 ± 1.4	34.9 ± 1.0	33.2 ± 1.8 (24–60)	13.3 ± 7.5	2.3 ± 1.8	0
Guppy, *Poecilia reticulata*	26.8 ± 1.4	23.1 ± 1.3	17.9 ± 0.3 (15–21)[a]	4.2 ± 0.8	2.6 ± 0.7	3.0 ± 1.2
Western mosquito fish, *Gambusia affinis*	33.7 ± 1.4	25.9 ± 0.8	21.6 ± 0.4 (19–25)	7.6 ± 1.5	5.3 ± 1.2	0
Largespring mosquito fish, *Gambusia geiseri*	31.9 ± 1.0	26.9 ± 1.1	20.8 ± 0.7 (17.5–28)	13.3 ± 4.1	3.5 ± 1.2	0
Least killifish, *Heterandria formosa*	25.8 ± 0.7	27.0 ± 0.9	13.5 ± 0.2 (12–14.5)	5.4 ± 2.0	3.1 ± 1.3	0
Delicate swordtail, *Xiphophorus cortezi*	35.1 ± 1.1	28.0 ± 1.4	30.0 ± 1.2 (21–35)[b]	2.9 ± 0.7	1.4 ± 0.7	3.5 ± 2.5
Variable platyfish, *Xiphophorus variatus* (Zarco)	25.7 ± 0.5	25.3 ± 0.5	25.6 ± 0.4 (23–27)	2.6 ± 1.1	1.0 ± 1.0	14.9 ± 3.1
Variable platyfish, *Xiphophorus variatus* (Encino)	24.6 ± 1.0	21.8 ± 0.3	24.7 ± 0.7 (20–28)	4.8 ± 2.7	5.6 ± 3.6	19.3 ± 10.1

[a] Total length 24.6 ± 0.3 mm; range of total length: 22–27 mm.

[b] Total length 44.1 ± 2.7 mm; range of total length: 26–58 mm.

mollies (*P. mexicana*) and *P. orri* we observed no aggressive behavior at all, and in all other species only very few cases of aggressive interactions were recorded (in 5–20% of the trials; data not shown).

Feeding experiment II: effect of a visually presented male

Feeding time reduction in poeciliid females could arise because females avoid male copulation attempts (i.e., the female is directly kept from feeding while the male approaches her from behind), but it could also arise because females are generally more vigilant in the presence of males (e.g., because females need to dedicate attention to monitor the males; see Discussion). In order to test this hypothesis, we performed an experiment similar to the previous one, but prevented the partner fish from physically interacting with the focal female. The test tank and testing conditions were identical to the first experiment. *Poecilia latipinna* collected at Lincoln Park (Brownsville, TX) and maintained as large, randomly outbred stocks in large (1000 l) seminatural pools in a greenhouse of the University of Oklahoma in Norman were used for this experiment.

The procedure for individual tests was identical to the one described for experiment I except that partner fish were confined within a transparent Plexiglas box ($30 \times 8 \times 8$ cm, open at the top and base) throughout the experiment. Again, each focal female experienced 2 subsequent experimental situations during which she could feed either with a male or with another female. We measured the time the focal female spent feeding from the presented stationary food source, from the surface of the water, the aquarium walls, and bottom, and on floating matter during the 5-min observation period.

Statistical analysis

We tested for overall differences in male mating activity (the sum of all sexual behaviors) across species/populations using analysis of covariance (ANCOVA; on log-transformed data), whereby "species identity" was a between-subjects factor. To test for an effect of male body size on male mating activity (e.g., Schlupp et al. 2001), male standard length was included as a covariate. To test for an effect of female body size (i.e., male mating preferences for large females, e.g., Plath et al. 2006), focal female standard length was included as another covariate. The interaction terms were not significant in the ANCOVA ($F < 0.71$, $P > 0.48$), suggesting that slope heterogeneity did not occur, and only the main effects were examined.

We also tested for differences among species in females' overall feeding motivation. The total time females spent feeding during both parts of a trial was compared among species using ANCOVA. Species identity was included as a between-subjects factor, and the focal female's standard length was included as a covariate. The interaction term was not significant ($F_{1,180} = 1.53$, $P = 0.14$), and only the main effects were analyzed.

Our main question was if females would spend less time feeding in the presence of a male and whether this feeding time reduction would differ among species. In one approach, we compared female feeding times (with a male or with a female) within each of the different species/populations using paired *t*-tests.

In another approach, we compared female feeding time reduction (determined as the relative time spent feeding in the presence of a male) across species. The relative time spent feeding with a male was calculated as follows: [time spent feeding with a male/(time spent feeding with female + time spent feeding with male)]. Hence, values <0.50 would indicate that the focal females fed less with a male partner, 0.50 would indicate no change at all and values > 0.50 would indicate that the females fed more with the male partner. All relative data were arcsine transformed for the analyses. We used ANCOVA, where species identity was a between-subjects factor. To account for differences in males' readiness to mate, male mating activity (individual values for the sum of all sexual behaviors) was included as a covariate. Because the overall feeding motivation of females might also influence the degree to which the females suffer from male harassment in terms of a feeding time reduction, the total feeding time of the focal females (feeding time with female + with male partner) was included as another covariate. For this analysis, we combined the data from both populations of the 2 species (*P. latipinna* and *X. variatus*) in which 2 different populations were investigated. There was no significant difference between populations in feeding time reduction (*P. latipinna*: Mann–Whitney *U* test: $U = 107.0$, $z = -0.51$, $P = 0.61$, $n_1 = 20$, $n_2 = 12$; *X. variatus*: 2-sample *t*-test on log-transformed data: $t_{18} = 0.55$, $P = 0.59$) or the number of male sexual behaviors (*P. latipinna*: 2-sample *t*-test on log-transformed data: $t_{30} = -0.59$, $P = 0.56$; *X. variatus*: 2-sample *t*-test: $t_{18} = -0.03$, $P = 0.98$). Because cave mollies have evolved under environmental conditions that differ vastly from those of typical surface populations (Parzefall 1969, 2001; Tobler et al. 2006) and an analysis of 10 polymorphic microsatellite loci revealed pronounced genetic differentiation from adjacent surface populations (Plath et al. 2007), surface-dwelling *P. mexicana* and cave mollies were not combined in this analysis but were statistically treated as different evolutionary entities. The interaction terms were not significant (ANCOVA: $F < 1.28$, $P > 0.25$), and only the main effects were analyzed.

We asked whether the proportion of courtship displays would affect female feeding times. Therefore, in subsequent analyses, only the subset of courting species was analyzed. First, we asked whether the ratio between sneak mating and courtship affects female feeding times on an individual level. Species identity was used as a between-subjects factor for an ANCOVA, in which the relative feeding time of the focal females with a male was the dependent variable. The total number of sexual behaviors and the fraction of courtship displays (number of courtship displays/total number of sexual behaviors) were included as covariates. In another analysis we asked if forced copulations would have a more severe effect on female feeding times than other sexual behaviors (i.e., nipping and courting). In this ANCOVA model, species identity was used as a factor, and the total number of sexual behaviors as well as the fraction of copulation attempts were used as covariates.

We also asked if females of species with more courting males are generally less disturbed by males (i.e., on a species, rather than on an individual level), such that we calculated the correlation between the mean fraction of courtship displays (mean values for each species) and the mean values for the time females spent feeding in the presence of a male using a nonparametric Kendall's correlation.

Generally, nonparametric tests were employed where the data deviated from normal distribution even after applying standard transformation methods, or where variances were unequal. All *P* values are 2-tailed.

RESULTS

Experiment I

Between-species comparison of male mating activity

There was a significant difference among species in the overall mating activity of males (sum of all sexual behaviors)

(ANCOVA: $F_{9,188} = 2.25$, $P = 0.021$). The mean (±SE) number of sexual behaviors per 5 min ranged from 7.8 ± 3.1 in *X. cortezi* to 33.8 ± 10.0 in surface-dwelling *P. mexicana* males (Table 2). Female body size ($F_{1,188} = 6.40$, $P = 0.012$) but not male size ($F_{1,188} = 0.93$, $P = 0.34$) had an effect, and males tended to direct more sexual behaviors toward larger females (post hoc Pearson correlation: $r_P = 0.13$, $P = 0.076$, $n = 200$).

Absolute feeding times of the focal females

The combined time females spent feeding with a male and with a female partner (as a measure of their overall motivation to feed) differed significantly among species (ANCOVA: $F_{9,189} = 16.01$, $P < 0.0001$; Table 1). The body size of the focal females also had an effect ($F_{1,189} = 5.98$, $P = 0.015$), and small females spent more time feeding than larger females (post hoc Pearson correlation: $r_P = -0.15$, $P = 0.037$, $n = 200$), suggesting that either large females are feeding more efficiently or they have a better starvation resistance. We checked if this result is different when only the time spent feeding in the presence of the partner female is considered; however, strikingly similar results were obtained (results not shown).

Female feeding time in the presence of a female or a male

Pairwise comparisons of female feeding times (absolute feeding times) revealed that females of all species/populations except cave molly females spent significantly less time feeding in the presence of a male compared with the time spent feeding with another female (Table 1).

Effect of male mating activity on females' feeding time

There was a significant difference between species in the relative times females spent feeding with a male (Table 3 and Figure 1). Post hoc pairwise comparisons revealed that the cave molly differed from all other groups (Fisher's protected least significant difference: $P < 0.02$), whereas all other post hoc comparisons were not significant ($P > 0.40$). When we omitted the cave molly from the analysis, the effect of the species term became nonsignificant ($F_{8,163} = 0.47$, $P = 0.88$). Male mating activity also had a significant effect (Table 3 and Figure 2). The total feeding time of the focal females did not affect the relative time spent feeding with a male (Table 3).

Table 3

ANCOVA on the relative time females spent feeding in the presence of a male as dependent variable

Effect	df	Mean square	*F*	*P*
Species identity	9	0.14	2.23	**0.022**
Total feeding time	1	0.14	2.29	0.13
Male mating activity	1	0.64	10.11	**0.0017**
Error	188	0.06		

The interaction terms were not significant ($F < 1.28$, $P > 0.25$), such that only the main effects were analyzed. Significant *P*-values ($P < 0.05$) are in bold typeface.

Comparison among courting and noncourting species

Courtship was observed only in *P. latipinna* (5 of $n = 32$ males), *P. reticulata* (12/20), and *X. cortezi* (6/12) and in *X. variatus* males (18/20; Table 1). We asked whether males of courting species would be generally less harassing to females (i.e., on a species level). When we correlated the mean values of the relative time focal females spent feeding in the presence of a male with the mean fraction of courtship displays across species, no significant correlation was detected (Kendall's $\tau = 0.18$, $z = 0.88$, $P = 0.38$, $n = 10$).

Male courtship and female feeding time

We also asked whether individual females would spend more time feeding when males are courting than in the presence of noncourting males while analyzing only the subset of courting species. In the ANCOVA, the interaction terms had no significant influence (Table 4), and only the main effects were analyzed. Species identity had no significant effect (Table 4). The total number of sexual behaviors had a highly significant effect, whereas the relative frequency of courtship displays did not significantly affect female feeding behavior (Table 4). Likewise, we asked if female feeding time reduction would be stronger when males show very high numbers of copulation attempts (gonopodial thrusts), but no effect of the relative frequency of copulation attempts was detected (Table 4).

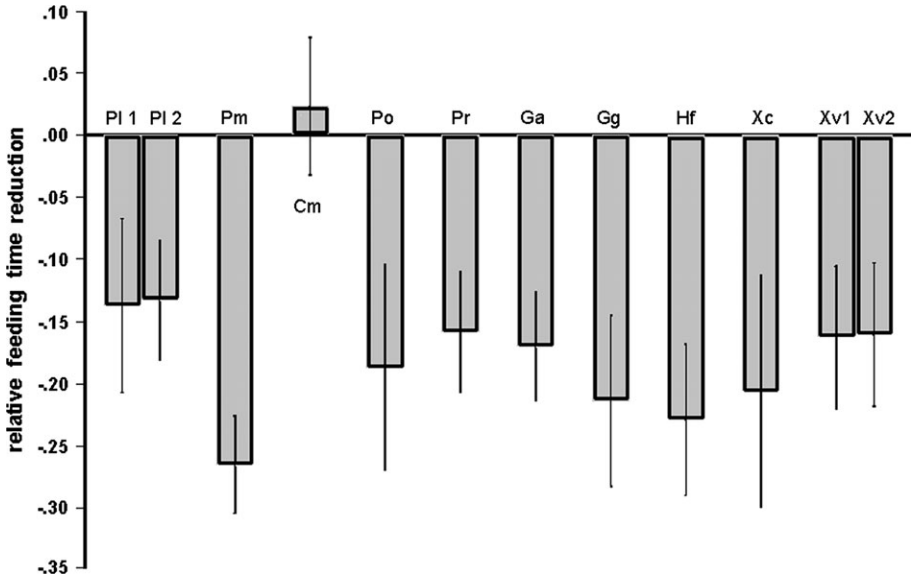

Figure 1

The relative feeding time reduction (mean ± SE) of females of the 9 species examined in the presence of a harassing male [(time spent feeding with a male/time spent feeding with a female + time feeding with a male) − 0.50]. Negative values indicate that the females spent less time feeding with a male than with a female. *Pl*, *Poecilia latipinna* (2 populations: *Pl1*, Lafayette; *Pl2*, Weslaco), *Pm*, *Poecilia mexicana*; *Cm*, cave molly (cave form of *P. mexicana*); *Po*, *Poecilia orri*; *Pr*, *Poecilia reticulata*; *Ga*, *Gambusia affinis*; *Gg*, *Gambusia geiseri*; *Hf*, *Heterandria formosa*; *Xc*, *Xiphophorus cortezi*; *Xv*, *Xiphophorus variatus* (2 populations: *Xv1*, Arroyo Zarco; *Xv2*, Arroyo Encino at Encino). Note that all but cave molly females experienced a strong feeding time reduction by male harassment.

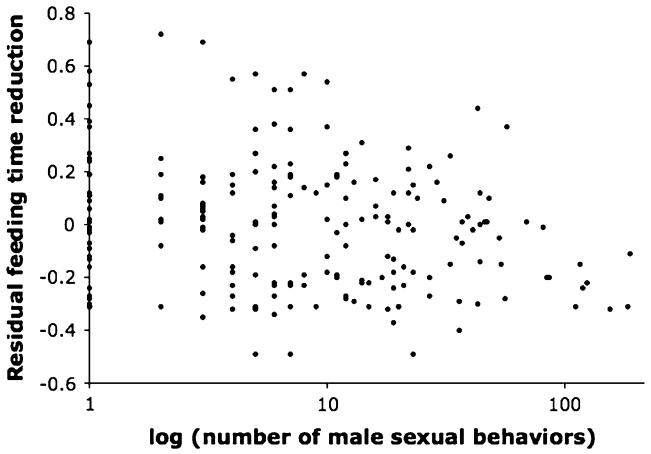

Figure 2
Scatter plot depicting the effect of male sexual activity on the reduction of females' feeding time. Residuals were obtained using an ANCOVA with "species identity" as independent variable and "total feeding time" as covariate (see Table 3).

Experiment II

Poecilia latipinna females spent significantly less time feeding in the presence of a male even when the male was presented only visually and physical interactions between the focal females and the partner fish were prevented (feeding time with partner female, mean ± SE: 85.9 ± 24.5 s; feeding time with male: 30.2 ± 9.9 s; paired *t*-test on log-transformed data: $t_{14} = 3.38$, $P = 0.005$). We calculated the relative time *P. latipinna* females spent feeding with a male (as a proportion of the total feeding time recorded). The fraction of time spent feeding with a male partner was determined as 35.9 ± 4.7% in experiment I (full contact) and 34.2 ± 9.2% in experiment II (visual presentation of the male). No statistically significant difference between the 2 experimental situations was detected (2-sample *t*-test: $t_{45} = -0.23$, $P = 0.82$).

Table 4
ANCOVA analyzing the relative effects of (a) the fraction of courtship displays and (b) the fraction of copulation attempts (gonopodial thrusts) on female feeding time reduction (i.e., the relative feeding time with male partner) in the subset of courting species

Effect	df	Mean square	*F*	*P*
(a) Courtship versus noncourtship				
Species identity	3	0.06	0.62	0.61
Male mating activity	1	0.66	6.99	**0.0099**
% Courtship	1	0.01	0.07	0.79
Error	78	0.10		
(b) Copulation attempts versus other sexual behaviors				
Species identity	3	0.08	0.79	0.50
Male mating activity	1	0.69	7.24	**0.0087**
% Copulation attempts	1	0.04	0.38	0.54
Error	78	0.10		

The interaction terms had no significant effect (*a*: $F < 2.55$, $P > 0.063$; *b*: $F < 0.54$, $P > 0.66$), and only the main effects were analyzed. Significant *P*-values ($P < 0.05$) are in bold typeface.

DISCUSSION

Male presence induced a significant shift in female feeding time allocation in all species studied except for the cave molly. Male visual presence was sufficient to cause this effect. The absence of a feeding time reduction in cave mollies confirms previous studies showing that sexual harassment in this population is basically absent (Plath et al. 2003, 2004). Cave molly males show very few sexual behaviors under natural conditions (Plath et al. 2005), so avoidance of male harassment likely plays a minor or even no role in the ecology of the cave molly.

Males in our study directed more sexual behaviors toward larger females. Male mating preferences for large female size are known from a variety of poeciliids (*G. holbrooki*: Bisazza et al. 1989; *P. latipinna*: Ptacek and Travis 1997; *P. mexicana*: Plath et al. 2006; *P. reticulata*: Dosen and Montgomerie 2004; Herdman et al. 2004) presumably because of their higher fecundity (e.g., *P. reticulata*: Herdman et al. 2004; *P. latipinna*: Tobler et al. 2005). Furthermore, large females may be easier targets for male gonopodial thrusts (mosquito fish, *G. holbrooki*: Pilastro et al. 1997).

Most importantly, female feeding time was generally lower in the presence of a male and decreased significantly with increasing male mating activity (sum of all sexual behaviors), but was not dependent on the kind of male mating behavior that males employed (courting or noncourting).

The results of the present study suggest that the degree of feeding time reduction between male size classes in *P. latipinna* (as described in another study [Schlupp et al. 2001]) is due to a decreased sexual activity in large males and not due to the presence of courtship in these males. In general, male mating activity per se (i.e., the sum of all sexual behaviors) determined the decline of female feeding time in the presence of males, whereas females did not feed more in the presence of males exhibiting courtship displays. It must be mentioned though that we detected no effect of male body size on the reduction of female feeding times in any of the species examined in the present study.

Comparisons of the effect of male harassment among different studies (e.g., between *P. latipinna* [Schlupp et al. 2001] and *G. holbrooki* [Pilastro et al. 2003]) may be problematic due to differences in the treatment of the test fish and/or the experimental design. This problem was avoided in the present study because all species/populations were tested under identical conditions. In our study, there was no significant difference in the amount of harassment between *P. latipinna* and *G. affinis* (a species closely related to *G. holbrooki*: Pyke 2006).

Why do poeciliid females show reduced feeding rates in the presence of a male? The general argument in the published literature is that nonreceptive poeciliid females avoid unwanted copulations (Houde 1997) and along with this any costs arising from multiple matings that may be detrimental to females' fitness. In many animals, male mating behavior can injure the female or even cause early death of the female (Rowe et al. 1994; Reale et al. 1996; Crudgington and Siva-Jothy 2000; Stutt and Siva-Jothy 2001; Blanckenhorn et al. 2002; Hosken et al. 2003; Morrow et al. 2003). Costs of mating have been shown to reduce female lifetime reproductive success throughout the animal kingdom (Civetta and Clark 2000; Rice 2000; Maklakov and Lubin 2004; Campbell 2005; Maklakov et al. 2005; Rönn et al. 2006). Damage of the female genital tract after copulations is also described for poeciliid fishes (Clark et al. 1954; Peters and Mäder 1964; Constanz 1984; Greven 2005). Along with the risk of genital damage, frequent mating (especially with more than one male) increases the risk of infection by sexually transmittable diseases (Able 1996). Thus, to avoid any potential costs imposed by unwanted copulations,

poeciliid females were hypothesized to direct attention toward males, thereby altering their time budget.

Our result that *P. latipinna* females experienced a very similar effect of a feeding time reduction even when the partner male was presented only visually and physical interactions (copulation attempts) were not possible (experiment II) underscores the importance of general shifts in female time budgets due to an increased vigilance in the presence of a male. Females may not only flee from actual copulation attempts but also appear to dedicate considerable time to monitoring surrounding males.

Feeding time reductions in females, however, may not only be caused by sexual harassment but also females may actually choose to interact with certain males. In such a case, female feeding time reductions would be a cost of female mate choice, that is, a trait under female control, rather than a cost that is imposed by males. Although female feeding time reduction is generally correlated with male sexual activity (experiment I), females may also direct attention toward a male if the male is unable to direct sexual behaviors toward the female (experiment II). Female feeding time reductions may therefore arise due to at least 2 factors, namely, male harassment (i.e., when the males exhibit a high frequency of sexual behaviors) and active female choice. For example, preferences for courting males were documented for some poeciliid species such as guppies (Farr 1980b; Kodric-Brown 1993; Kodric-Brown and Nicoletto 2001) and green swordtails (*Xiphophorus helleri*; Rosenthal et al. 1996), so that costs of female choice for courting males may actually mask the lower costs for females around courting males due to reduced sexual harassment. Both cases are not mutually exclusive but very difficult to tease apart experimentally because information about the female's motivation to interact with a male is hard to obtain. Future studies, in which females' motivations to feed or mate are manipulated or different response variables are considered, are thus highly warranted.

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